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THERAPEUTIC EFFICACY OF HIRUDO VERBANA LEECH EXTRACT IN A THERMAL WOUND MODEL

Actuality. *The ongoing environmental crisis, pandemics, and warfare, including the large-scale conflict in Ukraine, have led to a rise in wound-related conditions, burns, and inflammatory skin diseases. Oxidative stress is a key pathogenic factor delaying wound healing. There is a pressing need for new dermatological agents with antioxidant, reparative, and anti-inflammatory properties. Leech-derived products, long used in traditional medicine, are now being explored for these purposes, yet their efficacy in topical applications remains underinvestigated.*

The purpose of the work. To evaluate the reparative, anti-inflammatory, and antioxidant efficacy of a leech extract in a thermal burn wound model in rats, and to compare its effects to the reference drug Dioxizol.

Material and methods. *Hirudo verbana leeches were processed to obtain a sterile aqueous protein extract. Male Wistar rats with third-degree thermal burns were treated topically with either the leech extract, Dioxizol, or left untreated. Burn healing progression was assessed clinically and biochemically over 30 days. Serum concentrations of nitrotyrosine, IL-1β, and VEGF were measured via ELISA. Statistical significance was analyzed using Student's t-test and Mann–Whitney U test.*

Research results. *Leech extract significantly accelerated wound healing compared to controls and showed greater efficacy than Dioxizol in terms of granulation, epithelialization, and total healing time. Biochemical analysis revealed a 56% reduction in nitrotyrosine, a 70% decrease in IL-1β, and a 37,5% increase in VEGF levels, indicating strong antioxidant and reparative effects. The extract's effectiveness is likely due to bioactive proteins (hirudin, bdellin, antistasin) and compounds such as gibberellic acid.*

Conclusion. *Topical application of leech extract significantly improves burn wound healing through antioxidant, anti-inflammatory, and pro-regenerative mechanisms, outperforming the reference agent Dioxizol. These findings support further investigation into leech-based dermatological formulations.*

Key words: *leech extract, thermal wound, oxidative stress, nitrotyrosine, IL-1β, VEGF.*

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ТЕРАПЕВТИЧНА ЕФЕКТИВНІСТЬ ЕКСТРАКТУ П'ЯВКИ *HIRUDO VERBANA* В УМОВАХ МОДЕЛЮВАННЯ ОПІКОВОЇ РАНИ

Актуальність. *Екологічна криза, що триває, пандемії та війна, зокрема повномасштабний збройний конфлікт в Україні, призвели до зростання кількості поранень, опіків і запальних захворювань шкіри. Окислювальний стрес є ключовим патоге-*

нетичним чинником, що уповільнює загоєння ран. Існує нагальна потреба у створенні нових дерматологічних засобів з антиоксидантною, репаративною та протизапальною дією. Продукти, отримані з медичної п'явки, які давно використовуються в народній медицині, нині активно досліджуються, однак їхня ефективність за місцевого застосування залишається не досить вивченою.

***Мета дослідження** – оцінити репаративну, протизапальну й антиоксидантну ефективність екстракту з п'явок у моделі термічної опікової рани в щурів, порівняти її з ефектами референтного препарату Діоксизоль.*

***Матеріал і методи.** Медичні п'явки Hirudo verbana були використані для отримання стерильного водного білкового екстракту. Самців щурів лінії Вістар з термічними опіками III ступеня лікували місцево екстрактом п'явки, Діоксизолем або не лікували взагалі (контроль). Динаміку загоєння оцінювали клінічно та біохімічно протягом 30 днів. У сироватці крові вимірювали концентрацію нітротирозину, ІЛ-1β та VEGF методом ІФА. Статистичну обробку проводили з використанням t-критерію Стьюдента та U-критерію Манна – Вітні.*

***Результати дослідження.** Екстракт п'явки достовірно прискорював загоєння ран порівняно з контролем і перевершував Діоксизоль за показниками грануляції, епітелізації та загального часу загоєння. Біохімічний аналіз виявив зниження рівня нітротирозину на 56%, ІЛ-1β – на 70%, підвищення VEGF на 37,5%, що свідчить про потужну антиоксидантну та репаративну дію. Ефективність екстракту, імовірно, пов'язана з біоактивними білками (гірудин, бделін, антистазин) і сполуками, як-от гіберелінова кислота.*

***Висновок.** Місцеве застосування екстракту з п'явок достовірно покращує загоєння опікових ран завдяки антиоксидантним, протизапальним і репаративним механізмам, перевершує за ефективністю референтний препарат Діоксизоль. Отримані результати обґрунтовують доцільність подальших досліджень п'явкових дерматологічних засобів.*

***Ключові слова:** екстракт п'явки, термічна рана, окислювальний стрес, нітротирозин, ІЛ-1β, VEGF.*

Relevance. The second decade of the 21st century has been marked by a deterioration of the environmental situation, pandemics, accelerated global warming, heightened solar activity, and a large-scale conflict in Europe involving the invasion of Ukraine by the Russian Federation. These factors have contributed to an increase in mortality, not only due to various types of weaponry, but also due to a rise in diseases such as cardiovascular conditions, cancer, and other health disorders (Gobiet et al., 2014).

Stress, somatic pathologies, injuries, as well as thermal and chemical burns have resulted in a large number of military personnel and civilians requiring treatment for skin injuries and diseases (Singal & Lipner, 2023). There is an urgent need for the development and creation of new effective dermatological agents or topical dermatological formulations with reparative, anti-aging, anti-inflammatory, and antioxidant properties. Oxidative stress plays a significant role in the pathogenesis of the wound healing process. It is caused by the excessive production of reactive oxygen species (ROS) in biological systems, which cannot be completely neutralized by the endogenous antioxidant defense system. This condition reflects a disruption of redox homeostasis (Lopes et al., 2024; Vona et al., 2021). On the one hand, an excess of ROS directly damages lipids, proteins, and DNA, causing oxidative injury to the skin. The binding of ROS to DNA leads to the activation of proto-oncogenes, binding to proteins results in collagen degradation, and interaction with lipids causes lipid peroxidation and increased cell membrane permeability. Chemical damage to the skin and exposure to ultraviolet (UV) radiation also induce oxidative damage to macromolecules, leading to inflammation, photoaging, and various types of skin cancer.

On the other hand, excessive ROS participate in cellular signaling pathways by altering the expression of numerous genes. This contributes to impaired reparative skin regeneration, skin cell aging, and the development of inflammatory skin diseases such as psoriasis and dermatitis, as well as cancers such as melanoma and squamous cell carcinoma. ROS also mediate signaling pathways including MAPK, JAK/STAT, PI3K/AKT/mTOR, NF-κB, Nrf2, and SIRT1/FOXO, thereby influencing cytokine release and enzyme expression. These processes exacerbate skin inflammation, promote aging mechanisms, and delay wound healing (Liu et al., 2023; Jaffri, 2023; Su et al., 2019). An increased level of ROS that exceeds the capacity of the antioxidant defense system leads to chronic inflammation. This chronic inflammation, in turn, can cause collagen fragmentation, disorganization of collagen fibers, and disruption of skin cell functions, thereby contributing to the development of various skin diseases. The use of topical and oral antioxidants may provide dermatologists with a safe and effective means to modulate oxidative processes (Kruk & Duchnik, 2014). Currently, there are several antioxidants used for optimizing reparative skin regeneration during wound healing, treating skin cell aging caused by oxidative stress, inflammation, and cancer. The most commonly applied are alpha-tocopherol and tocotrienols, ascorbic acid, carotenoids, bioflavonoids, lipoic acid, ubiquinone, selenium compounds, and glutathione, as well as niacinamide, also known as vitamin B3, and thiotriazoline (Papaccio et al., 2022; Belenichev et al., 2024c).

Among synthetic antioxidants used in surgery and dermatology, thiotriazoline and ethylmethylhydroxypyridine succinate (Mexidol) are notable as potent antioxidants. Antioxidants of animal origin have also found application. These include egg white proteins such as

ovotransferrin and phosvitins. Additionally, antioxidant peptides are well known – hydrolysates of proteins derived from milk, fish, eggs, meat, and by-products (heads, skin, fins, intestines, blood) (Rathnapala et al., 2021; Sarmadi & Ismail, 2010; Addor, 2017).

Antioxidants of animal origin reduce oxidative modification of proteins by inhibiting Fe^{2+}-dependent initiation of free radical processes and normalizing the thiol-disulfide system, thereby exhibiting wound-healing activity (Corino & Rossi, 2021; Abeyrathne et al., 2022). Antioxidants derived from leeches are of great interest for the treatment of wounds of various origins. Components of leech extract with antioxidant activity, such as hirudin, bdellin, eglin, calin, and hyaluronidase, contribute to fibrinolytic, anti-inflammatory, vasodilatory, and wound-healing effects. Additionally, leech extract components exhibit immunomodulatory, antimicrobial, and anticancer activities (Bilden et al., 2025).

In international practice, oral preparations of leech extract – Zhixiong capsules and Shenyuandan capsules – are used as antioxidants in the treatment of cardiovascular diseases (Li et al., 2022). To date, the efficacy of topical application of leech extract in skin injuries of chemical and physical origin, as well as in inflammatory and immune-mediated skin diseases and wounds, has not been thoroughly studied. The antimicrobial properties of this antioxidant, particularly the feasibility of its combination with known antimicrobial agents, require further investigation. The above considerations underscore the relevance of the present study.

Materials and methods. Preparation of medical leeches. The medicinal leeches were maintained using the modern jar method. They were bred at the educational-scientific-research laboratory of cellular and organismal biotechnology at Zaporizhzhia National University (TU U 05.0-02125243-002:2009 "Medicinal Leech", sanitary-epidemiological opinion of the Ministry of Health of Ukraine № 05.03.02-06/49982, dated 12.08.2009).

Technology for Preparing Leech Extract. Medicinal leeches Hirudo verbana weighing 0,8–1,1 g were selected. They were dried using sterile filter paper, fragmented, washed twice in 0,9% sodium chloride physiological solution, and ground in crushed sterile glass with the addition of a small amount of physiological solution. After grinding, physiological solution was added at a mass ratio of 1:10. The mixture was extracted in a refrigerator at a temperature of +6 °C to +8 °C, centrifuged at a speed of no more than 4 000 g for 40 minutes, and filtered through disposable sterile Millipore membrane filters with a pore diameter of 0,2 μm. The protein concentration was determined using the pyrogallol red method on a Beckman Coulter AU480 analyzer (the protein concentration was 0,486 g/L). The sterility of the extract was checked by inoculating it on microbiological nutrient meat-peptone agar to detect the presence of bacteria or fungi.

The extract was applied to the wound surface using compresses. For this, 2 ml of extract was diluted in 2 ml of distilled water. From this solution, 0,5 ml was applied to the wound surface on the day of injury, as well as on days 1, 3, 7, 10, and 14.

Laboratory Animals. The experimental studies were conducted in accordance with the Order of the Ministry of Health of Ukraine № 944 dated December 14, 2009, "Procedure for Preclinical Study of Medicinal Products and Examination of Preclinical Study Materials". The research was performed on an adequate number of experimental animals, and all manipulations were carried out following the regulations on the use of animals in biomedical research (Strasbourg, 1986, with amendments made in 1998), the "General Ethical Principles of Animal Experimentation" (Kyiv, 2001), which are consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes". Prior to the commencement of this study, the Bioethics Committee of Zaporizhzhia State Medical and Pharmaceutical University reviewed and approved the study protocols as well as procedures related to animal housing and their use in the experiments (protocol № 6 dated November 12, 2024).

White nonlinear male rats weighing 260–370 g, obtained from the vivarium of the State Institution "Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine", were used in the experiments. Before the experiment, each animal was examined by a qualified veterinarian to assess its health status. Subsequently, the animals underwent a 10-day quarantine period and were randomly assigned to groups depending on the dose of the administered drug.

The animals were housed in polycarbonate cages measuring 550 × 320 × 180 mm with galvanized steel lids measuring 660 × 370 × 140 mm and equipped with glass drinking bottles. No more than five rats were kept in each cage. Each cage was labeled with the study number, species, sex, animal identification numbers, and dose information. The cages were placed on racks according to dose levels and cage numbers indicated on the labels. The animal housing room was maintained under the following conditions: temperature 20–24 °C, humidity 30–70%, and a 12-hour light/12-hour dark cycle (Belenichev et al., 2024a). All rats were provided ad libitum with a standard laboratory animal diet supplied by the

company “Phoenix”, Ukraine. Drinking water from the municipal water supply (subjected to reverse osmosis and UV sterilization) was given without restrictions. Alder (Alnus glutinosa) wood shavings, previously sterilized by autoclaving, were used as bedding. Animals in the cages were marked with gentian violet.

Thermal wound model. Before inducing the burn, the fur was carefully shaved on an area of 3 cm × 3 cm. Prior to modeling the thermal wound, rats were anesthetized with sodium thiopental (PJSC “Kyivmedpreparat”, Ukraine) at a dose of 40 mg/kg administered intraperitoneally. The thermal wound was induced by applying a copper plate with an area of 23 cm², heated by a nickel coil to 100 °C, for 5 seconds. This procedure resulted in the development of third-degree burns of types 3a and 3b. Due to its high thermal conductivity, the copper plate can dissipate heat deeper into the skin tissue, creating a full-thickness burn. Histological analysis reveals an attenuated epidermis with coagulative damage extending through the entire dermis and subcutaneous tissue. Furthermore, this model is representative of clinical situations observed in hospitalized burn patients, such as impaired immune regulation and bacterial infections. The model is capable of reproducing systemic bacterial infections caused by both Gram-positive and Gram-negative bacteria (Sharma et al., 2022). Animals in the first group were treated by applying leech extract. Animals in the second group received applications of the reference drug “Dioxizol” (JSC “Pharmaceutical Firm Darnitsa”, Ukraine). The third group served as untreated controls. The fourth group consisted of intact (healthy) rats. The medicinal formulations were applied immediately after the burn and then daily throughout the entire experiment.

Blood collection and preparation of biological material. Immediately after the completion of the experiment, under anesthesia (sodium thiopental, 40 mg/kg), blood was collected from the abdominal aorta of the animals using a syringe and transferred into special tubes. The blood was centrifuged at +4 °C at 1 500 rpm for 20 minutes using an Eppendorf 5804R centrifuge. The serum was collected and stored at −60 °C.

Enzyme-Linked Immunosorbent Assay (ELISA). Interleukin IL-1β in blood serum was measured as a marker of inflammation using a solid-phase enzyme-linked immunosorbent assay with the IL-1β ELISA KIT № KE10003 (Proteintech, USA). Nitrotyrosine was determined in blood serum as a marker of oxidative stress and to evaluate the antioxidant effects of the tested drugs using a solid-phase ELISA with the NITROTYROSINE ELISA KIT № HK501 (Hycult Biotech, USA). Vascular endothelial growth factor (VEGF) in blood serum was measured as a marker of skin reparative regeneration using a solid-phase ELISA with the Rat VEGF ELISA Kit № ab100786 (Abcam, USA). All assays were performed on a microplate ELISA reader Sirio-S (Seac Radim Company, Italy).

Statistical analysis of the obtained results. The numerical experimental data were processed using variation statistics with the aid of a personal computer AMD Athlon XP and licensed software STATISTICA 6.1 (serial number AGAR 909 E415822FA). The arithmetic mean (M) and standard error of the mean (±m) were calculated. The significance of the obtained results was assessed using the parametric Student’s t test and the non-parametric Mann – Whitney U test.

Results of the study. The assessment of burn wound treatment results, presented in Table 1, indicates that compared to untreated animals, both the tested leech extract and the reference drug Dioxizol demonstrated a pronounced wound healing effect, accelerating the regeneration processes. The evaluation of the burn eschar rejection rate showed that the activity of the compared agents was higher relative to the control group. A similar trend was observed in other indicators of therapeutic efficacy.

It should be noted that for a number of parameters, the studied leech extract was significantly more effective compared to the control group, and in terms of the timing of granulation appearance, onset of marginal epithelialization, and complete healing time of the thermal wound, it surpassed Dioxizol. Table 2 presents the results of complete healing of burn wounds at various

Table 1

Criteria for the efficacy of the tested agents in burn wounds: healing time of burn wounds in rats (days)

Experimental groups	Resolution of perifocal reaction	Rejecting scab	Granulation appearance	Start of marginal epithelialization	Complete healing (full epithelia-lization)
Control (Thermal wound) (n = 10)	5,7 ± 2,0	13,1 ± 1,7	18,4 ± 2,2	19,0 ± 2,0	47,7 ± 4,0
Thermal wound + Leech extract (n = 10)	1,7 ± 0,7*[1]	6,3 ± 1,0*	8,0 ± 1,2*	11,3 ± 1,4*	28,0 ± 2,1*[1]
Thermal wound + Dioxizol (n = 10)	2,8 ± 0,9	7,0 ± 1,0*	10,1 ± 2,0*	15,1 ± 1,5*	33,7 ± 2,4*

* – $p < 0,05$ compared to the control group;

[1] – $p < 0,05$ compared to the dioxizol group.

observation time points. By day 25, full epithelialization of burn wounds occurred in 60% of rats treated with the leech extract, 50% of rats treated with Dioxizol, and 10% of untreated rats. By day 30 of treatment, these values were 80, 60, and 30%, respectively.

Table 2

Percentage of completely epithelialized burn wounds with the use of the tested agents

Experimental groups	Day 25	Day 30
Control (Thermal wound) (n = 10)	10%	30%
Thermal wound + Leech extract (n = 10)	60%	80%
Thermal wound + Dioxizol (n = 10)	50%	60%

The above indicates a higher therapeutic effect. As shown in Table 3, after pathology modeling, oxidative stress reactions are significantly activated, as evidenced by an 8-fold increase in nitrotyrosine levels in the serum of rats with burn wounds. Additionally, the burn wound model leads to a pronounced surge in inflammatory responses. We observed an 11,7-fold increase in the pro-inflammatory cytokine IL-1β in the serum of rats with burn wounds. Moreover, VEGF expression in the blood of rats with burn wounds increased by 34,4% by day 30 of the experiment, indicating the initiation of the burn healing process.

Course administration of leech extract led to a 56% reduction in the oxidative stress marker nitrotyrosine and a 70% decrease in the inflammatory marker IL-1β. Additionally, the course application of leech extract resulted in a 37,5% increase in VEGF expression compared to the control group. Regarding the degree of nitrotyrosine and IL-1β reduction and VEGF elevation, leech extract significantly outperformed the similar effects of Dioxizal ointment. The Dioxizal ointment showed a significant effect only on the reduction of IL-1β. These results further confirm the pronounced wound-healing, antioxidant, and anti-inflammatory effects of the leech extract.

Discussion of results. The obtained results from the thermal burn wound model are consistent with other studies that have demonstrated an increase in VEGF during the proliferative phase of wound healing. VEGF stimulates various cell types to cooperate in restoring the epidermal and dermal layers of tissue and drives the proliferative phase of healing, ultimately leading to complete wound closure. In the context of burn injuries, VEGF levels are often elevated in the serum of burn patients, indicating its crucial role in reparative processes following burn trauma. Studies have also shown that VEGF levels correlate with both local and systemic tissue edema in burn patients, further highlighting its involvement in vascular changes during burn wound healing. VEGF is generally considered beneficial for wound repair (Abdulazeem et al., 2022).

IL-1β is significantly elevated in burn wounds and plays a crucial role in the mechanisms of secondary damage to the skin and other target organs. Interaction of IL-1β with its receptors activates nuclear transcription factors AP-1 and NF-κB, which alter the behavior of target cells, leading to the development of an acute-phase cellular response, expression of other pro-inflammatory mediators, stimulation of astrocyte expression of inducible nitric oxide synthase (iNOS) and cytotoxic nitric oxide derivatives, increased mitochondrial pore permeability, and initiation of apoptosis (Cai et al., 2019).

IL-1β-induced hyperexpression of nitric oxide (NO) in burn wounds leads to the activation of oxidative and nitrosative stress and damage to the protein structures of the skin, thereby slowing the processes of reparative regeneration. In burn wounds, the presence and increase of nitrotyrosine serve as indicators of oxidative stress and the production of reactive nitrogen species, such as peroxynitrite and nitrosonium ion. Nitrotyrosine levels are often elevated both in burned skin and in the blood and other organs following burn injury (Oliveira et al., 2004; Belenichev et al., 2024b). The mechanism of the observed effects of the leech extract is likely related to its chemical composition. Several researchers have shown that leech saliva and other parts may contain compounds such as 1,2-benzisothiazol-3-amine,

Table 3

Concentration of molecular markers in serum after burn wound on day 30 of the experiment

Experimental Groups	Nitrotyrosine, ng/ml	IL-1β, pg/ml	VEGF, pg/ml
Intact	0,91 ± 0,041	0,32 ± 0,021	72,3 ± 3,2
Control (Thermal wound) (n = 10)	7,32 ± 0,32[1]	3,77 ± 0,075[1]	97,2 ± 6,7[1]
Thermal wound + Leech extract (n = 10)	3,22 ± 0,12*[12]	1,11 ± 0,042*[12]	133,7 ± 10,2*[12]
Thermal wound + Dioxizol (n = 10)	6,83 ± 0,33[1]	2,53 ± 0,074*[1]	94,8 ± 8,2[1]

* – $p < 0,05$ compared to the control group;

[1] – $p < 0,05$ compared to the intact group;

[2] – $p < 0,05$ compared to the Dioxizol group.

2,6-di-tert-butyl-4-methylene-2,5-cyclohexadienone, and various hydrocarbons. The leech extract may also contain different biologically active proteins that can similarly contribute to its anticancer and anti-inflammatory effects. Active proteins such as hirustasin and bdelins can influence transcription factors and reduce caspase-dependent apoptosis. Through the NF-κB pathway, proteins in the leech extract may regulate the expression of VEGF (Bouïs et al., 2006; Colwell et al., 2005). This explains the high wound-healing activity of the leech extract revealed in our study. Hirudotherapy and the use of leech extract–based preparations can significantly increase VEGF expression in wounds. VEGF is a potent growth factor that stimulates the formation of new blood vessels, which is crucial for tissue restoration, wound healing, and various other biological processes. This suggests that leech proteins may play a role in stimulating angiogenesis, which is necessary for wound repair.

The antioxidant activity of the leech extract identified in our study may be associated with gibberellic acid. Several studies have demonstrated that gibberellic acid plays a role in regulating antioxidant defense mechanisms by increasing the activity of key enzymes such as superoxide dismutase, ascorbate peroxidase, and glutathione reductase, thereby reducing cellular oxidative damage. Gibberellic acid is a factor that stimulates tissue growth and repair (Iftikhar et al., 2019; Asif et al., 2022). Gibberellic acid demonstrated a high affinity for the anti-apoptotic protein Bcl-2 in docking analysis (Bilden et al., 2025). Antistasin is also an active compound isolated from leeches, exhibiting both anticoagulant and anti-inflammatory effects. Leeches contain proteins that interact with IL-1β and thereby may interrupt IL-1β-dependent inflammatory responses, apoptosis, and oxidative stress (Sig et al., 2017; Van Den Eeckhout et al., 2021).

Conclusions. Thus, for the first time, we have demonstrated the therapeutic effect of leech extract in an experimental burn wound model, aimed at accelerating reparative skin regeneration. The mechanism underlying this effect of leech extract involves reparative, anti-inflammatory, and antioxidant pathways. In terms of efficacy, the leech extract surpasses the reference drug, Dioxizole ointment. The obtained results justify the promise of further investigation of leech extract.

Prospects for further research: we plan to conduct a more detailed study of the antioxidant mechanism of leech extract in various wounds – specifically, its effects on oxidative modifications of proteins and lipids, as well as its influence on the expression of key antioxidant defense enzymes such as Cu/Zn-SOD, GPR-1, and GPR-4.

BIBLIOGRAPHY

Abdulazeem L., Tariq A., Abdalkareem Jasim S. Effects of vascular endothelial growth factor (VEGFR-1 and VEGFR-2) in burn wound healing. Archives of Razi Institute. 2022. № 77 (2). P. 747–751. DOI: 10.22092/ARI.2022.356981.1954.

Abeyrathne E. D. N. S., Nam K., Huang X., Ahn D. U. Plant- and animal-based antioxidants' structure, efficacy, mechanisms, and applications : A review. Antioxidants (Basel). 2022. № 11(5). P. 1025. DOI: 10.3390/antiox11051025.

Addor F. A. S. Antioxidants in dermatology. Anais Brasileiros de Dermatologia. 2017. № 92 (3). P. 356–362. DOI: 10.1590/abd1806-4841.20175697.

Asif S., Jang Y.-H., Kim E.-G., Jan R., Asaf S., Aaqil Khan M., Farooq M., Lubna, Kim N., Lee I.-J., et al. The role of exogenous gibberellic acid and methyl jasmonate against white-backed planthopper (Sogatella furcifera) stress in rice (Oryza sativa L.). International Journal of Molecular Sciences. 2022. № 23. P. 14737. DOI: 10.3390/ijms232314737.

Belenichev I., Bukhtiyarova N., Ryzhenko V., Makyeyeva L., Morozova O., Oksenych V., Kamyshnyi O. Methodological approaches to experimental evaluation of neuroprotective action of potential drugs. International Journal of Molecular Sciences. 2024. № 25 (19). P. 10475. DOI: 10.3390/ijms251910475.

Belenichev I., Popazova O., Bukhtiyarova N., Savchenko D., Oksenych V., Kamyshnyi O. Modulating nitric oxide: Implications for cytotoxicity and cytoprotection. Antioxidants (Basel). 2024. № 13 (5). P. 504. DOI: 10.3390/antiox13050504.

Belenichev I., Ryzhenko V., Popazova O., Bukhtiyarova N., Gorchakova N., Oksenych V., Kamyshnyi O. Optimization of the search for neuroprotectors among bioflavonoids. Pharmaceuticals (Basel). 2024. № 17 (7). P. 877. DOI: 10.3390/ph17070877.

Bilden A., Sabancılar İ., Yalçın Azarkan S., Karadağlı K., Kaya S., Kahraman M., Çiçek M. Investigating the therapeutic potential of crude leech saliva based on its anticancer, antioxidant, and anti-inflammatory effects. Current Issues in Molecular Biology. 2025. № 47. P. 328. DOI: 10.3390/cimb47050328.

Bouïs D., Kusumanto Y., Meijer C., Mulder N. H., Hospers G. A. A review on pro- and anti-angiogenic factors as targets of clinical intervention. *Pharmacological Research*. 2006. № 53 (2). P. 89–103. DOI: 10.1016/j.phrs.2005.10.006.

Cai Y., Xue F., Quan C., Qu M., Liu N., Zhang Y., Fleming C., Hu X., Zhang H. G., Weichselbaum R., Fu Y. X., Tieri D., Rouchka E. C., Zheng J., Yan J. A critical role of the IL-1β–IL-1R signaling pathway in skin inflammation and psoriasis pathogenesis. *Journal of Investigative Dermatology*. 2019. № 139 (1). P. 146–156. DOI: 10.1016/j.jid.2018.07.025.

Colwell A. S., Beanes S. R., Soo C., Dang C., Ting K., Longaker M. T., Atkinson J. B., Lorenz H. P. Increased angiogenesis and expression of vascular endothelial growth factor during scarless repair. *Plastic and Reconstructive Surgery*. 2005. № 115 (1). P. 204–212.

Corino C., Rossi R. Antioxidants in animal nutrition. *Antioxidants (Basel)*. 2021. № 10 (12). P. 1877. DOI: 10.3390/antiox10121877.

Gobiet A., Kotlarski S., Beniston M., Heinrich G., Rajczak J., Stoffel M. 21st century climate change in the European Alps : A review. *Science of The Total Environment*. 2014. № 493. P. 1138–1151. DOI: 10.1016/j.scitotenv.2013.07.050.

Iftikhar A., Ali S., Yasmeen T., Arif M. S., Zubair M., Rizwan M., Alhaithloul H. A. S., Alayafi A. A. M., Soliman M. H. Effect of gibberellic acid on growth, photosynthesis and antioxidant defense system of wheat under zinc oxide nanoparticle stress. *Environmental Pollution*. 2019. № 254 (Pt B). P. 113109. DOI: 10.1016/j.envpol.2019.113109.

Jaffri M. Reactive oxygen species and antioxidant system in selected skin disorders. *Malaysian Journal of Medical Sciences*. 2023. № 30 (1). P. 7–20. DOI: 10.21315/mjms2023.30.1.2.

Kruk J., Duchnik E. Oxidative stress and skin diseases: Possible role of physical activity. *Asian Pacific Journal of Cancer Prevention*. 2014. № 15 (2). P. 561–568. DOI: 10.7314/apjcp.2014.15.2.561.

Li X., Zhang Y., Liu H. X., Shang J. J., Zhou Q., Li A. Y., Lai X. L., Xing W. L., Jia S. H. A randomized, placebo-controlled, double-blind trial to evaluate efficacy and safety of Shen-Yuan-Dan capsules, a traditional Chinese medicine, for treatment of peri-procedure myocardial injury following percutaneous coronary intervention. *Complementary Therapies in Medicine*. 2022. № 69. P. 102841. DOI: 10.1016/j.ctim.2022.102841.

Liu H.-M., Cheng M.-Y., Xun M.-H., Zhao Z.-W., Zhang Y., Tang W., Cheng J., Ni J., Wang W. Possible mechanisms of oxidative stress-induced skin cellular senescence, inflammation, and cancer and the therapeutic potential of plant polyphenols. *International Journal of Molecular Sciences*. 2023. № 24 (4). P. 3755. DOI: 10.3390/ijms24043755.

Lopes F. B., Sarandy M. M., Novaes R. D., Valacchi G., Gonçalves R. V. OxInflammatory responses in the wound healing process : A systematic review. *Antioxidants (Basel)*. 2024. № 13 (7). P. 823. DOI: 10.3390/antiox13070823.

Oliveira G. V., Shimoda K., Enkhbaatar P., Jodoin J., Burke A. S., Chinkes D. L., Hawkins H. K., Herndon D. N., Traber L., Traber D., Murakami K. Skin nitric oxide and its metabolites are increased in nonburned skin after thermal injuries. *Shock*. 2004. № 22 (3). P. 278–282. DOI: 10.1097/01.shk.0000135259.90311.33.

Papaccio F., D'Arino A., Caputo S., Bellei B. Focus on the contribution of oxidative stress in skin aging. *Antioxidants (Basel)*. 2022. № 11 (6). P. 1121. DOI: 10.3390/antiox11061121.

Rathnapala E. C. N., Ahn D. U., Abeyrathne S. Functional properties of ovotransferrin from chicken egg white and its derived peptides : A review. *Food Science and Biotechnology*. 2021. № 30 (5). P. 619–630. DOI: 10.1007/s10068-021-00901-3.

Sarmadi B. H., Ismail A. Antioxidative peptides from food proteins : A review. *Peptides*. 2010. № 31 (10). P. 1949–1956. DOI: 10.1016/j.peptides.2010.06.020.

Sharma R., Yeshwante S., Vallé Q., Hussein M., Thombare V., McCann S. M., Maile R., Li J., Velkov T., Rao G. Rat burn model to study full-thickness cutaneous thermal burn and infection. *Journal of Visualized Experiments*. 2022. 186. DOI: 10.3791/64345.

Sig A. K., Guney M., Uskudar Guclu A., Ozmen E. Medicinal leech therapy – an overall perspective. *Integrative Medicine Research*. 2017. № 6 (4). P. 337–343. DOI: 10.1016/j.imr.2017.08.001.

Singal A., Lipner S. R. A review of skin disease in military soldiers: challenges and potential solutions. *Annals of Medicine*. 2023. № 55 (2). P. 2267425. DOI: 10.1080/07853890.2023.2267425.

Su L. J., Zhang J. H., Gomez H., Murugan R., Hong X., Xu D., Jiang F., Peng Z. Y. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative Medicine and Cellular Longevity*. 2019. P. 5080843. DOI: 10.1155/2019/5080843.

Van Den Eeckhout B., Tavernier J., Gerlo S. Interleukin-1 as innate mediator of T cell immunity. *Frontiers in Immunology*. 2021. № 11. P. 621931. DOI: 10.3389/fimmu.2020.621931.

Vona R., Pallotta L., Cappelletti M., Severi C., Matarrese P. The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. *Antioxidants*. 2021. № 10 (2). P. 201. DOI: 10.3390/antiox10020201.

REFERENCES

Abdulazeem, L., Tariq, A., & Abdalkareem Jasim, S. (2022). An investigation of vascular endothelial growth factor (VEGFR-1 and VEGFR-2) in burn wound healing. *Archives of Razi Institute*, 77 (2), 747–751. DOI: 10.22092/ARI.2022.356981.1954.

Abeyrathne, E.D.N.S., Nam, K., Huang, X., & Ahn, D.U. (2022). Plant- and animal-based antioxidants' structure, efficacy, mechanisms, and applications: A review. *Antioxidants (Basel)*, 11 (5), 1025. DOI: 10.3390/antiox11051025.

Addor, F.A.S. (2017). Antioxidants in dermatology. *Anais Brasileiros de Dermatologia*, 92 (3), 356–362. DOI: 10.1590/abd1806-4841.20175697.

Asif, S., Jang, Y.-H., Kim, E.-G., Jan, R., Asaf, S., Aaqil Khan, M., Farooq, M., Lubna, Kim, N., & Lee, I.-J., et al. (2022). The role of exogenous gibberellic acid and methyl jasmonate against white-backed planthopper (Sogatella furcifera) stress in rice (Oryza sativa L.). *International Journal of Molecular Sciences*, 23, 14737. DOI: 10.3390/ijms232314737.

Belenichev, I., Bukhtiyarova, N., Ryzhenko, V., Makyeyeva, L., Morozova, O., Oksenych, V., & Kamyshnyi, O. (2024a). Methodological approaches to experimental evaluation of neuroprotective action of potential drugs. *International Journal of Molecular Sciences*, 25 (19), 10475. DOI: 10.3390/ijms251910475.

Belenichev, I., Popazova, O., Bukhtiyarova, N., Savchenko, D., Oksenych, V., & Kamyshnyi, O. (2024b). Modulating nitric oxide: Implications for cytotoxicity and cytoprotection. *Antioxidants (Basel)*, 13 (5), 504. DOI: 10.3390/antiox13050504.

Belenichev, I., Ryzhenko, V., Popazova, O., Bukhtiyarova, N., Gorchakova, N., Oksenych, V., & Kamyshnyi, O. (2024c). Optimization of the search for neuroprotectors among bioflavonoids. *Pharmaceuticals (Basel)*, 17 (7), 877. DOI: 10.3390/ph17070877.

Bilden, A., Sabancılar, İ., Yalçın Azarkan, S., Karadağlı, K., Kaya, S., Kahraman, M., & Çiçek, M. (2025). Investigating the therapeutic potential of crude leech saliva based on its anticancer, antioxidant, and anti-inflammatory effects. *Current Issues in Molecular Biology*, 47, 328. DOI: 10.3390/cimb47050328.

Bouïs, D., Kusumanto, Y., Meijer, C., Mulder, N.H., & Hospers, G.A. (2006). A review on pro- and anti-angiogenic factors as targets of clinical intervention. *Pharmacological Research*, 53 (2), 89–103. DOI: 10.1016/j.phrs.2005.10.006.

Cai, Y., Xue, F., Quan, C., Qu, M., Liu, N., Zhang, Y., Fleming, C., Hu, X., Zhang, H.G., Weichselbaum, R., Fu, Y.X., Tieri, D., Rouchka, E.C., Zheng, J., & Yan, J. (2019). A critical role of the IL-1β-IL-1R signaling pathway in skin inflammation and psoriasis pathogenesis. *Journal of Investigative Dermatology*, 139 (1), 146–156. DOI: 10.1016/j.jid.2018.07.025.

Colwell, A.S., Beanes, S.R., Soo, C., Dang, C., Ting, K., Longaker, M.T., Atkinson, J.B., & Lorenz, H.P. (2005). Increased angiogenesis and expression of vascular endothelial growth factor during scarless repair. *Plastic and Reconstructive Surgery*, 115 (1), 204–212.

Corino, C., & Rossi, R. (2021). Antioxidants in animal nutrition. *Antioxidants (Basel)*, 10 (12), 1877. DOI: 10.3390/antiox10121877.

Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., & Stoffel, M. (2014). 21st century climate change in the European Alps – A review. *Science of The Total Environment*, 493, 1138–1151. DOI: 10.1016/j.scitotenv.2013.07.050.

Iftikhar, A., Ali, S., Yasmeen, T., Arif, M.S., Zubair, M., Rizwan, M., Alhaithloul, H.A.S., Alayafi, A.A.M., & Soliman, M.H. (2019). Effect of gibberellic acid on growth, photosynthesis and antioxidant defense system of wheat under zinc oxide nanoparticle stress. *Environmental Pollution*, 254 (Pt B), 113109. DOI: 10.1016/j.envpol.2019.113109.

Jaffri, M. (2023). Reactive oxygen species and antioxidant system in selected skin disorders. *Malaysian Journal of Medical Sciences*, 30 (1), 7–20. DOI: 10.21315/mjms2023.30.1.2.

Kruk, J., & Duchnik, E. (2014). Oxidative stress and skin diseases: Possible role of physical activity. *Asian Pacific Journal of Cancer Prevention*, 15 (2), 561–568. DOI: 10.7314/apjcp.2014.15.2.561.

Li, X., Zhang, Y., Liu, H.X., Shang, J.J., Zhou, Q., Li, A.Y., Lai, X.L., Xing, W.L., & Jia, S.H. (2022). A randomized, placebo-controlled, double-blind trial to evaluate efficacy and safety of Shen-Yuan-Dan capsules, a traditional Chinese medicine, for treatment of peri-procedure myocardial injury following percutaneous coronary intervention. *Complementary Therapies in Medicine*, 69, 102841. DOI: 10.1016/j.ctim.2022.102841.

Liu, H.-M., Cheng, M.-Y., Xun, M.-H., Zhao, Z.-W., Zhang, Y., Tang, W., Cheng, J., Ni, J., & Wang, W. (2023). Possible mechanisms of oxidative stress-induced skin cellular senescence, inflammation, and cancer and the therapeutic potential of plant polyphenols. *International Journal of Molecular Sciences*, 24 (4), 3755. DOI: 10.3390/ijms24043755.

Lopes, F.B., Sarandy, M.M., Novaes, R.D., Valacchi, G., & Gonçalves, R.V. (2024). OxInflammatory responses in the wound healing process: A systematic review. *Antioxidants (Basel)*, 13 (7), 823. DOI: 10.3390/antiox13070823.

Oliveira, G.V., Shimoda, K., Enkhbaatar, P., Jodoin, J., Burke, A.S., Chinkes, D.L., Hawkins, H.K., Herndon, D.N., Traber, L., Traber, D., & Murakami, K. (2004). Skin nitric oxide and its metabolites are increased in nonburned skin after thermal injuries. *Shock*, 22 (3), 278–282. DOI: 10.1097/01.shk.0000135259.90311.33.

Papaccio, F., D'Arino, A., Caputo, S., & Bellei, B. (2022). Focus on the contribution of oxidative stress in skin aging. *Antioxidants (Basel)*, 11 (6), 1121. DOI: 10.3390/antiox11061121.

Rathnapala, E.C.N., Ahn, D.U., & Abeyrathne, S. (2021). Functional properties of ovotransferrin from chicken egg white and its derived peptides: A review. *Food Science and Biotechnology*, 30 (5), 619–630. DOI: 10.1007/s10068-021-00901-3.

Sarmadi, B.H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides*, 31 (10), 1949–1956. DOI: 10.1016/j.peptides.2010.06.020.

Sharma, R., Yeshwante, S., Vallé, Q., Hussein, M., Thombare, V., McCann, S.M., Maile, R., Li, J., Velkov, T., & Rao, G. (2022). Rat burn model to study full-thickness cutaneous thermal burn and infection. *Journal of Visualized Experiments*, 186. DOI: 10.3791/64345.

Sig, A.K., Guney, M., Uskudar Guclu, A., & Ozmen, E. (2017). Medicinal leech therapy – an overall perspective. *Integrative Medicine Research*, 6 (4), 337–343. DOI: 10.1016/j.imr.2017.08.001.

Singal, A., & Lipner, S.R. (2023). A review of skin disease in military soldiers: challenges and potential solutions. *Annals of Medicine*, 55 (2), 2267425. DOI: 10.1080/07853890.2023.2267425.

Su, L.J., Zhang, J.H., Gomez, H., Murugan, R., Hong, X., Xu, D., Jiang, F., & Peng, Z.Y. (2019). Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxidative Medicine and Cellular Longevity*, 2019, 5080843. DOI: 10.1155/2019/5080843.

Van Den Eeckhout, B., Tavernier, J., & Gerlo, S. (2021). Interleukin-1 as innate mediator of T cell immunity. *Frontiers in Immunology*, 11, 621931. DOI: 10.3389/fimmu.2020.621931.

Vona, R., Pallotta, L., Cappelletti, M., Severi, C., & Matarrese, P. (2021). The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. *Antioxidants*, 10 (2), 201. DOI: 10.3390/antiox10020201.

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Contribution of the authors:

Popazova O.O. – concept and design of the study, literature review, experimental work, data collection, writing and editing of the manuscript, statistical analysis, interpretation of results, formulation of conclusions;

Aminov R.F. – development of the research methodology, literature review, drafting and revising the manuscript, interpretation of findings, approval of the final version;

Ryzhenko O.I. – participation in experimental design, data acquisition, experimental work, literature analysis, contribution to writing, statistical processing of data, interpretation of research outcomes;

Makyeyeva L.V. – coordination of research activities, collection and analysis of scientific sources, writing and proofreading the manuscript, visualization of results, preparation of conclusions;

Filianskyi I.O. – literature research, drafting of the manuscript, statistical evaluation, interpretation of experimental data;

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